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γ-Secretase-Regulated Signaling Mechanisms: Notch and Amyloid Precursor Protein

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1. Introduction

In *Drosophila*, Notch mutations lost a lateral signaling ability and produced a neurogenic phenotype, where cells destined to become epidermis switch fate and give rise to neural tissue (Artavanis-Tsakonas *et al.* 1995; Lewis 1998). Therefore, when Notch signaling was disrupted, too many neurons were generated. Notch attracted further interest because sel-12, which appears to facilitate the reception of signaling mediated by lin-12 (*C. elegans* Notch), was identified by screening for a suppressor of lin-12 gain-of-function mutation (Levitan and Greenwald 1995). Since sel-12 is thought to be a counterpart of human presenilin (PS), which is a catalytic component of γ-secretase and has been implicated in Alzheimer's disease (AD), it was thought that the Notch signaling pathway might have a close relation with AD. Thus, many scientists have investigated the relationship between Notch signaling and AD. As we focused below, it has become clear that the Notch signaling pathway is controlled by γ-secretase-mediated proteolysis.

Both Notch receptors and their ligands are evolutionally conserved single transmembrane-spanning proteins (type 1 transmembrane protein; amino terminus is extracellular and carboxyl terminus is cytoplasmic.) that control the fates of numerous cells in both invertebrates and vertebrates (Artavanis-Tsakonas *et al.* 1995; Artavanis-Tsakonas *et al.* 1999; Justice and Jan 2002). For example, Delta, a major Notch ligand, expressing cells inhibit the neural determination of neighboring Notch-expressing neural stem cells (NSCs) during neurogenesis (Nakayama *et al.* 2008a). In addition, it is well known that isoforms of Notch mediate somitogenesis, differentiation of lymphoid cells as well as differentiation of NSCs, and that dysregulation of Notch signaling causes developmental defects or cancer in mammals (Bolos *et al.* 2007).

The molecular mechanism of Notch signaling is quite unique in that it is controlled by proteolytic cleavage reactions (Artavanis-Tsakonas *et al.* 1999; Justice and Jan 2002). In the canonical Notch signaling pathway, ligands bind to the extracellular domain of Notch on

neighboring cells, and trigger sequential proteolytic cleavage. Finally, the intracellular domain (ICD) of Notch (NICD) is released from the cell membrane by γ-secretase and translocates to the nucleus to modulate gene expression through binding to transcription factors. Therefore, γ-secretase plays a central regulatory role in Notch signaling. First, we give a detailed interpretation of Notch itself and Notch signaling as well as its role in differentiation of NSCs.

The Notch signaling pathway has long been believed to be mono-directional because ligands for Notch were generally considered unable to transmit signals into the cells expressing them (Fitzgerald and Greenwald 1995; Henderson *et al.* 1997). However, several groups have shown that Delta is cleaved sequentially by proteases, probably including ADAM and γ-secretase (Ikeuchi and Sisodia 2003; LaVoie and Selkoe 2003; Six *et al.* 2003), and ICD of Delta is released from the cell membrane and translocates to the nucleus (LaVoie and Selkoe 2003; Six *et al.* 2003). We have also shown that ICD of mouse Delta binds to Smads, which are transcription factors for TGF-β/Activin signaling pathway, and enhances transcription of specific genes required for neuronal differentiation (Hiratochi *et al.* 2007). These results suggest that Delta also has a signaling mechanism similar to Notch signaling. Thus, we also review this issue that the Notch-Delta signaling pathway is bi-directional and similar mechanisms regulated by γ-secretase are involved in both directions of the Notch-Delta signaling pathway in developing NSCs.

γ-Secretase was first identified as a protease that cleaves amyloid precursor protein (APP) within the transmembrane (TM) domain and produces Aβ peptides (Haass and Selkoe 1993), which are thought to be pathogenic in AD (Hardy 1997; Selkoe 2001). However, the physiological functions of γ-secretase have not been clarified (Kopan and Ilagan 2004; Selkoe and Wolfe 2007). Recently, it was demonstrated that more than 50 type 1 transmembrane proteins, including APP, Notch and Delta, are substrates for γ-secretase (McCarthy *et al.* 2009) and their ICDs are also released from the cell membrane, similar to Notch. These observations that the common enzyme, γ-secretase, modulates proteolysis and the turnover of putative signaling molecules have led to the attractive hypothesis that mechanisms similar to the Notch signaling pathway may contribute widely to γ-secretase-regulated signaling pathways (Koo and Kopan 2004; Nakayama *et al.* 2008a; Nakayama *et al.* 2011).

Interestingly, it has also been reported that ICD of APP (AICD), which is released from the cell membrane by γ-secretase, translocates to the nucleus (Cupers *et al.* 2001; Gao and Pimplikar 2001; Kimberly *et al.* 2001) and may function as a transcriptional regulator (Cao and Sudhof 2001; Guenette 2002). As the apoptotic potential of AICD has been demonstrated, it is likely that APP signaling induces cell death, which leads to AD.

To explore APP signaling, we established embryonic carcinoma P19 cell lines overexpressing AICD (Nakayama *et al.* 2008b). Although neurons were differentiated from these cell lines with all-*trans*-retinoic acid (RA) treatment, AICD expression induced neuron-specific apoptosis. The effects of AICD were restricted to neurons, with no effects observed on non-neural cells. Furthermore, we evaluated changes in gene expression induced by AICD during this process of neuron-specific cell death using DNA microarrays (Ohkawara *et al.* 2011). The results of microarray analysis indicated that AICD induces dynamic changes in the gene expression profile. Therefore, it is likely that APP also has a signaling mechanism and that AICD may play a role in APP signaling, which leads to AD.

Here, we focus on molecular mechanisms of the Notch-Delta signaling pathway in a bi-directional manner and discuss the possibility that γ-secretase-regulated mechanisms similar to the Notch-Delta signaling pathway may play a potential role in signaling events involving type 1 transmembrane proteins. In addition, we introduce the current topics of γ-secretase. We also discuss the possibility that APP signaling induces dynamic changes in gene expression, which may be closely correlated with AICD-induced neuron-specific apoptosis, leading to AD.

2. Notch

2.1 Notch and its ligands

The typical Notch gene encodes a 300-kD type 1 transmembrane protein with the large extracellular domain which contains about 36 tandem epidermal growth factor (EGF)-like repeats (Wharton *et al.* 1985). The 11th and 12th EGF-like repeats are necessary and sufficient for binding to its ligands in *Drosophila* (Rebay *et al*. 1991). NICD is also large and has six tandem ankyrin-like (CDC10) repeats (Wharton *et al.* 1985). The fundamental structures are well conserved throughout evolution, although the numbers of EGF-like repeats vary from 10 in *C. elegans* (Glp-1) (Yochem and Greenwald 1989) to 36 in *Drosophila* and some vertebrate Notch .

While *Drosophila* has only one Notch gene, four Notch isoforms (Notch1 to 4) have been found in mammals. TAN1 (Notch1), which is a first identified mammalian homolog of Notch, was cloned as a gene responsible for human T cell acute lymphoblastic leukemia (T-ALL) (Ellisen *et al.* 1991). Notch2 was also cloned as an oncogene of cat thymic lymphoma (Rohn *et al.* 1996). A mutation of the Notch3 gene causes cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (Joutel *et al.* 1996), in which the main symptom is cerebral vascular disorder. Interestingly, Notch4 is a cellular counterpart of the oncogene of mouse mammary tumor virus (int3) and expresses in vascular endothelial cells (Sarkar *et al.* 1994).

While *Drosophila* has two different ligands Delta (Kopczynski *et al.* 1988) and Serrate (Fleming *et al.* 1990), two families of ligands, Delta family (Delta-like protein: Dll1, 3 and 4) (Bettenhausen *et al.* 1995; Dunwoodie *et al.* 1997; Shutter *et al.* 2000) and Jagged family (Jagged1 and 2) (Lindsell *et al.* 1995; Shawber *et al.* 1996), have been identified in mammals to date. *C. elegans* has two ligands, Lag-2 (Tax *et al.* 1994) and Apx-1 (Mello *et al.* 1994). The extracellular domains of all these ligands also contain variable numbers of EGF-like repeats; for example *Drosophila* Delta has nine, most vertebrate Deltas have eight, and *C. elegans* Lag-2 has two repeats. All of these ligands also share a single copy of a second cysteine-rich conservative motif called the DSL (Delta: Serrate: Lag-2) domain (Tax *et al.* 1994), which is essential for binding to Notch (Henderson *et al.* 1997). In addition, spondylocostal dysostosis (SCD), which is characterized by abnormal vertebral segmentation, is caused by mutations of Dll3 gene (Sparrow *et al.* 2002). Alagille syndrome, which is a multi-system disorder characterized by paucity of bile ducts and congenital heart disease, is associated with a Jagged1 mutation (Oda *et al.* 1997) as well as a Notch2 mutation (McCright *et al.* 2002).

ICDs of all ligands are relatively short compared to those of Notch and it was thought that none of ICDs of Notch ligands display any significant sequence similarity throughout

evolution (Henderson *et al.* 1994). As described below, structural evidence supports the idea that ICDs of these ligands are non-functional. However, we have revealed that Delta homologues display significant sequence similarity, which is restricted to vertebrates, in their ICDs (Hiratochi *et al.* 2007). There is no homology between these vertebrate Deltas and *Drosophila* Delta. In addition, Dll3, a divergent type of Delta, does not show any homology to other Delta in ICD.

2.2 The molecular mechanism of the Notch signaling pathway

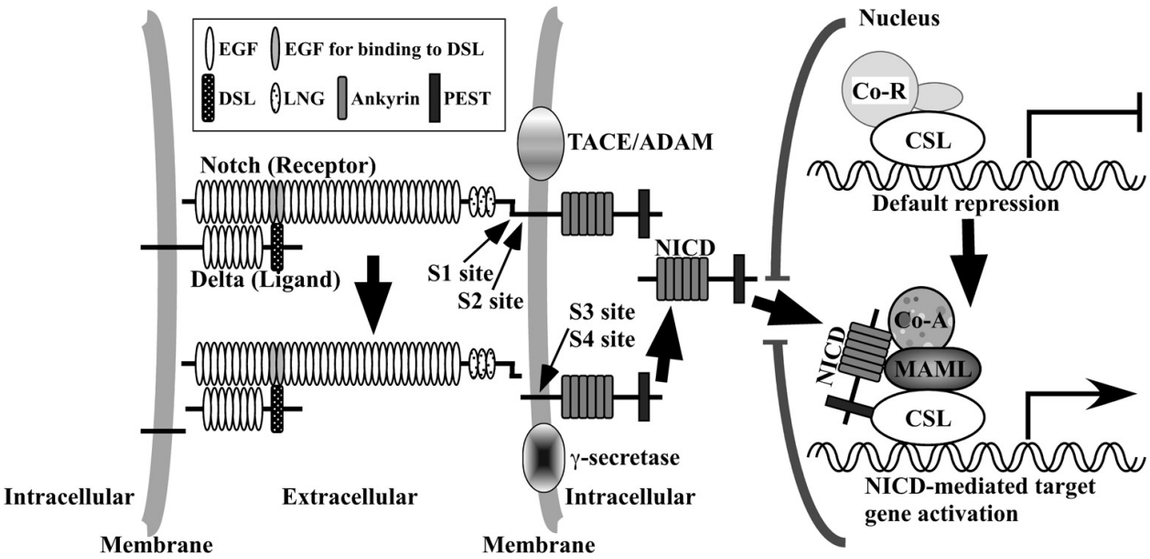
Fig. 1 shows a diagram of the Notch signaling pathway. In the canonical Notch signaling pathway, ligands bind to the extracellular domain of Notch on neighboring cells. Both Notch (Gupta-Rossi *et al.* 2004) and its ligands (Itoh *et al.* 2003) undergo ubiquitin-regulated internalization. Mind bomb (Mib) is essential for efficient activation of Notch signaling in this step. Mib is a RING-type E3 ubiquitin ligase that ubiquitylates ICDs of Notch ligands and promotes internalization of these ligands in a ubiquitination-dependent manner (Itoh *et al.* 2003). As a result of these reactions, conformations of Notch and its ligands may be changed by pulling each other to trigger sequential proteolytic cleavages called the regulated intramembrane proteolysis (RIP) mechanism (Brown *et al.* 2000). The RIP mechanism requires sequential cleavage steps to occur within the juxtamembrane (JM) and TM domains, and these steps are carried out by metalloproteases and γ-secretase, respectively (Selkoe and Kopan 2003). Since precise steps of Notch processing are recently made clear and those steps are very similar to that of APP, we mentioned about details of these processes in "3.2 Processing mechanisms of several γ-secretase substrates, such as APP, are very similar to that of Notch."

Finally, γ-secretase serves to release NICD from the cell membrane to the cytoplasm and released NICD translocates to the nucleus. Thus, γ-secretase plays a central role in the regulation of Notch signaling. Although NICD has a nuclear localization signal and is accumulated in the nucleus as an activated form of Notch, mechanisms of the transport of NICD from cytoplasm to nucleus have not yet been clarified.

In the nucleus, NICD binds to transcription factors and controls expressions of certain genes. Members of the CSL family (CBF1/RBP-jκ in mammals, Su(H) in *Drosophila* and Lag-1 in *C. elegans*) are major downstream transcription factors of Notch signaling (Artavanis-Tsakonas *et al.* 1995; Kimble and Simpson 1997; Artavanis-Tsakonas *et al.* 1999). NICD binds to CSL transcription factors; six tandem ankyrin-like repeats lying in NICD are essential for binding to CSL transcriptional factors (Roehl *et al.* 1996). As NICD also binds to Mastermind-like proteins (MAML family in mammals) (Wu *et al.* 2000), the CSL-NICD-MAML complex is formed. As a result of forming these complexes, co-repressors are dispersed from CSL and co-activators such as P/CAF and P300 are recruited by these complexes (Wallberg *et al.* 2002). Therefore, the function of CSL complexes is converted from a transcriptional repressor to an activator. Finally, activated CSL complexes bind to the *cis*-acting DNA sequences of target genes and enhance the transcriptional activity of these genes.

The most established target genes for Notch signaling are Hes (Hairy/Enhancer of split in *Drosophila*) genes, which code for the basic helix-loop-helix (bHLH) transcriptional repressor for tissue-specific genes (Kageyama *et al.* 2007). Seven mammalian Hes, designated Hes1 to Hes7, have been identified to date, although the mouse does not have Hes4. Hes1 and Hes5

bind to their target DNA sequences called N box (CACNAG) by forming homodimers or heterodimers with Hey (Hes-related with YRPW motif) 1 or Hey2, and to recruit histone deacetylase (HDAC) activity by associating with Groucho, resulting in transcriptional repression (Akazawa *et al.* 1992; Leimeister *et al.* 1999; Iso *et al.* 2001). Moreover, they associate with E proteins which are ubiquitously expressed bHLH factors and prevent proneural bHLH factors, such as Neurogenin, from forming functional complexes with E protein (Kageyama *et al.* 2007). In this manner, Notch represses the differentiation of cells to specific lineages. In addition, Delta expression is induced by proneural genes that code for bHLH transcriptional factor, although multiple POU-binding factors are also important for Delta expression in mammalian NSCs (Nakayama *et al.* 2004). Thus, Notch signaling strongly inhibits Delta expression.



Notch proteins are expressed on the cell surface as heterodimers after cleavage at the S1 site by furin. The binding of Notch to the ligand triggers sequential proteolytic cleavage of RIP. When Notch binds to the ligand, Notch is cleaved at the S2 site in the juxtamembrane region by TACE or ADAM protease. Next, the remaining protein stub is further cleaved by γ-secretase at the S3 and S4 sites within the transmembrane domain and NICD is released from the membrane. Then, NICD translocates into the nucleus and binds to the CSL together with MAML. The resultant CSL–NICD–MAML complex removes co-repressors from CSL transcription factor and recruits a co-activator, resulting in conversion from repressor to activator. Finally, the complexes of CSL-NICD-MAML-co-activators promote transcription of the target genes

Fig. 1. Notch signaling pathway.

2.3 Notch signaling in the differentiation of NSCs

The definition of NSCs is that cells can self-renew and are capable of differentiating into main phenotypes of the nervous system, such as neurons, astrocytes and oligodendrocytes. In mammals, such cells have been isolated from the developing neural tube and more recently from the adult brain. Although there are some data showing that Notch signaling plays a role in the adult NSCs, a large majority of evidence for Notch signaling in controlling NSCs differentiation comes from analysis of embryonic neurogenesis.

In the developing mammalian central nervous system, NSCs repeat self-renewal by symmetric cell division to increase the total number of NSCs as a first step (NSCs/progenitor cells expansion phase). In this phase, Notch signaling is thought to maintain those NSCs in the proliferating and undifferentiating state (Fortini 2009; Kopan and Ilagan 2009; Pierfelice *et al.* 2011). Recently, it has been shown that expression of Hes1, the target genes for Notch signaling, oscillates with a period of about 2 hours in this phase (Kageyama *et al.* 2007). Hes1 expression may induce the oscillatory expression of Dll1 gene and the proneural Neurogenin2 (Ngn2) gene by periodic repression. Thus, concentrations of Dll1 mRNA and both Ngn2 mRNA and protein also oscillate with an inverse correlation with Hes1 (Kageyama *et al.* 2008; Shimojo *et al.* 2011). It is thought that Ngn2 cannot induce differentiation of neuron when Ngn2 expression oscillated and Dll1 leads to the activation of Notch signaling to maintain NSCs in proliferating state.

In next phase (neurogenic phase), NSCs undergo asymmetric cell division, where each NSC divides into two distinct types, NSC and neuron. In this phase, oscillatory expressions of Hes1 disappear (Kageyama *et al.* 2008; Shimojo *et al.* 2011). Since Hes1 expression is repressed, Dll1 and Ngn2 are constitutively expressed in a sustained manner and Ngn2 induces neuronal differentiation. Although the role of Notch signaling is not well understood, numb, which is an antagonist of Notch signaling (Frise *et al.* 1996; Guo *et al.* 1996; Spana and Doe 1996), is thought to be a critical component of NSCs asymmetrical division. During NSC divisions in this phase, numb appeared to be asymmetrically distributed to the neuronal daughter cells and was absent in undifferentiated NSCs (Zhong *et al.* 1996; Zhong *et al.* 1997; Chenn 2005). Thus, these observations suggest that numb inhibits Notch signaling and promotes differentiation to neuron in the neuronal daughter cells. After the generation of neurons, NSCs differentiate into oligodendrocytes and ependymal cells, followed by differentiation into astrocytes (gliogenic phase).

Recently, it has been shown that Notch signaling may also play an essential role in maintenance and differentiation of adult NSCs (Imayoshi and Kageyama 2011). Usually, adult NSCs are in the dormant state (quiescent state) and Notch signaling may maintain this state of adult NSCs. It is thought that NSCs turn from dormant state into dividing state, when the activity of Notch signaling falls down. Thus, Notch signaling controls the balance between dormant state and differentiation state of adult NSCs.

2.4 Delta signaling may be involved in neuronal differentiation

The Notch signaling pathway has long been thought to be mono-directional because ligands for Notch were generally considered unable to transmit signals into cells expressing these ligands (Henderson *et al.* 1994; Fitzgerald and Greenwald 1995). Indeed, it was thought that none of ICDs of putative Notch ligands display any significant sequence similarity throughout evolution (Henderson *et al.* 1994). Moreover, replacement of most of ICD of LAG-2, a *C. elegans* lin-12 (Notch) ligand, with a β-galactosidase fusion protein has no discernible effect on LAG-2 function (Henderson *et al.* 1994). In contrast, however, it has been reported that the extracellular domain of Notch expressed in the mesoderm provided a positive signal to the overlaying ectoderm in *Drosophila* as mentioned below (Baker and Schubiger 1996). Since these observations suggest that signaling in the opposite direction also exists, the important and critical question is whether signaling events occur not only from ligand-expressing cells to Notch-expressing cells but also vice versa, i.e., in a bi-directional manner.

Recently, evidence has been accumulating in support of a functional role of ICD of Notch ligands, which implies the existence of bi-directional signaling mechanisms. For example, Delta has been shown to release ICD from the cell membrane when cleaved by ADAM protease and γ-secretase (Qi *et al.* 1999; Ikeuchi and Sisodia 2003; LaVoie and Selkoe 2003; Six *et al.* 2003). Several groups have reported evidences supporting the nuclear localization of Delta ICD (Bland *et al.* 2003; LaVoie and Selkoe 2003; Six *et al.* 2003). These observations suggest that Delta ICD is released from the cell membrane by RIP. Indeed, we have shown that Delta homologues display significant sequence similarity, which is restricted to vertebrates, in their ICDs (Hiratochi *et al.* 2007). It is likely that conservation of these amino acid sequences reflect the functional importance of Delta ICD.

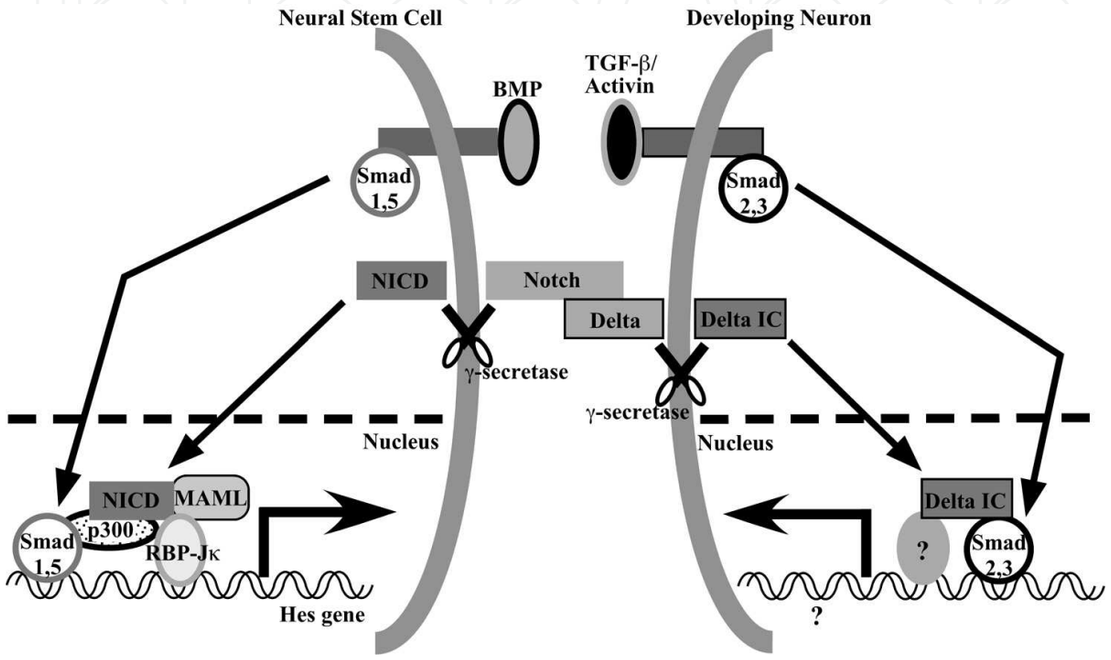
To clarify the question of whether the Notch-Delta signaling pathway is bi-directional, we investigated the effect of Notch on differentiation of NSCs isolated from mouse embryos (Hiratochi *et al.* 2007). When NSCs were co-cultured on a monolayer of mouse Dll1-expressing COS7 cells, the rate at which neurons emerged was lower than that in controls. As mentioned above, Notch signaling maintains the proliferating and undifferentiating state of NSCs and inhibits the differentiation into neurons (Fortini 2009; Kopan and Ilagan 2009; Pierfelice *et al.* 2011). Therefore, these observations indicate that Dll1 on COS7 cells generates signals to neighboring NSCs that express Notch and thus activates Notch signaling. Conversely, when NSCs were co-cultured on a monolayer of mouse Notch1-expressing COS7 cells, the rate of neurons developing from NSCs was significantly higher than that in control cultures. These results suggest that Notch1 on COS7 cells may also generate signals to neighboring NSCs and these ligands, probably Delta, may then transmit signals into cells expressing them to promote neuronal differentiation. Thus, signaling events may occur not only from Delta-expressing cells to Notch-expressing cells but also vice versa, that is, in a bi-directional manner, during differentiation of NSCs. Indeed, Baker and Schubiger published results from a mosaic experiment in *Drosophila*, which showed that expression of Notch in the mesoderm of Notch mutant suppressed the ectodermal defects of this mutant (Baker and Schubiger 1996). This effect was inferred to be due to the extracellular domain of the protein and not its signaling function, since activated Notch failed to produce non-autonomous suppression (Baker and Schubiger 1996). These results indicate that the extracellular domain of Notch expressed in the mesoderm sent a positive signal to the overlying ectoderm. Thus, these observations further support the hypothesis that Notch-expressing cells also send a signal to Delta-expressing cells.

2.5 Delta ICD may modify expression of certain genes

The nuclear localization of Delta ICD suggests that this domain may have effects on the transcription of a specific target gene similar to NICD. To examine this possibility, we searched for transcriptional factors capable of binding to ICD of Dll1 (Dll1IC) using a new method and identified Smads as a Dll1IC binding transcription factor through the differentiation process of mouse NSCs (Hiratochi *et al.* 2007).

Smads are transcription factors and have been shown to act as mediators of signaling by the TGF-β superfamily. Eight Smads, designated Smad1 to Smad8, have been identified to date in mammal (Miyazawa *et al.* 2002; Derynck and Zhang 2003). Smad2 and Smad3 are activated by TGF-β and activin (Eppert *et al.* 1996; Zhang *et al.* 1996; Nakao *et al.* 1997), while

Smad1 and Smad5 are major components that are activated by bone morphogenic proteins (BMPs) (Hoodless *et al.* 1996; Kretzschmar *et al.* 1997; Suzuki *et al.* 1997). Although Smad1 and Smad5 did not bind to Dll1IC, Smad2 and Smad3 showed strong binding (Hiratochi *et al.* 2007). These observations indicate that Dll1IC can modify TGF-β/Activin signaling through binding to Smad2 and/or Smad3. However, BMP signaling, which is known to inhibit neurogenesis and to enhance the appearance of astrocytes, may not be affected by Dll1IC, because Dll1IC did not bind to Smad1 or Smad5.



Notch receptor also generates signals to Delta expressed on the surface of neighboring NSCs. Delta is cleaved sequentially by proteases, probably including ADAM and γ-secretase, and finally the intracellular domain of Delta (DeltaIC) is released from the cell membrane and translocates to the nucleus, where it mediates TGF-β/Activin signaling through binding to Smad2/3 and enhances transcription of specific genes leading to neuronal differentiation. It is well known that NICD is also released from the cell membrane by proteases similar to the ones involved in the cleaving of Delta, then translocates to the nucleus to modulate gene expression through binding to the transcription factor, that is, Suppressor of Hairless (Su(H), RBP-jκ in mammals) together with MAML. This means that similar mechanisms are involved in both directions of the bi-directional Notch-Delta signaling pathway. BMPs, another group belonging to the TGF-β superfamily, have recently been shown to inhibit neurogenesis and to enhance the generation of astrocytes from NSCs. It has also been demonstrated that NICD and activated Smad1/5 form a complex with p300 in the specific promoter sequence, which contains both the RBP-jκ and Smad binding sequences. It is therefore possible that the TGF-β superfamily mediates both neurogenesis and gliogenesis from NSCs coupled with the bi-directional Notch-Delta signaling pathway.

Fig. 2. Schematic of the bi-directional model of Notch-Delta signaling pathway in the process of NSC differentiation.

Although we have yet to determine the actual target genes for the Dll1IC-Smad complex, we showed that binding of Dll1IC to Smad enhanced its transcriptional activity using the 9XCAGA-Luc promoter-reporter system that responds specifically to Smad3 (Dennler *et al.* 1998; Jonk *et al.* 1998), as a model system. These results strongly suggest that Dll1IC

mediates transcription of certain genes, which are targets of TGF-β/Activin signaling, through binding to Smad2 and/or Smad3.

As mentioned above, it is likely that Delta transmits signals into NSCs expressing them to promote neuronal differentiation. To test this possibility, we established embryonic carcinoma P19 cells stably overexpressing Dll1IC (Hiratochi *et al.* 2007). Although control P19 cells have been shown to be induced to differentiate into neurons, RA stimulation is essential for the induction of neurons from these P19 cells. However, neurons could be induced from P19 cells stably overexpressing Dll1IC without RA stimulation and this induction was strongly inhibited by SB431542, a specific inhibitor of TGF-β type1 receptor (Laping *et al.* 2002) that activates Smad2 and Smad3. These results suggest that overexpression of Dll1IC in P19 cells induced neurons through binding to Smad2 and/or Smad3. Therefore, it is highly possible that Delta signaling also plays an important role in neuronal differentiation. Recently, it has been reported that TGF-β inhibits proliferation and accelerates differentiation of the hippocampal granule neuron (Lu *et al.* 2005). This observation also supports our hypothesis.

A schematic model of Notch-Delta signaling pathway in the process of NSC differentiation is shown in Fig. 2.

3. γ-Secretase

3.1 Overview of γ-secretase

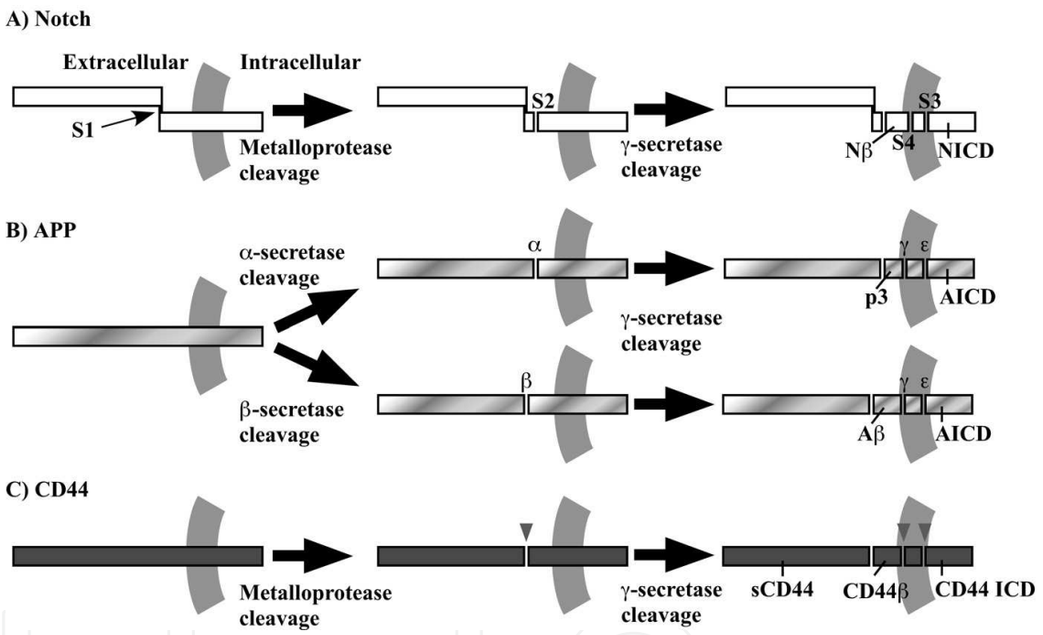
γ-Secretase was first identified as a protease that cleaves APP within the TM domain and produces Aβ peptides (Haass and Selkoe 1993), which are thought to have pathogenic roles in AD. However, the physiological functions of this enzyme have not been clarified (Kopan and Ilagan 2004; Selkoe and Wolfe 2007). The γ-secretase is a complex composed of PS, nicastrin (NCT), anterior pharynx defective-1 (Aph-1), and PS enhancer-2 protein (Pen-2) (Iwatsubo 2004; Kopan and Ilagan 2004; Selkoe and Wolfe 2007). PS is a catalytic component of the γ-secretase complex, and the two PS genes, PS1 gene (*PSEN1*) (Sherrington *et al.* 1995) located on chromosome 14 and PS2 gene (*PSEN2*) (Levy-Lahad *et al.* 1995; Rogaev *et al.* 1995) located on chromosome 1, were identified by genetic linkage analyses as the genes responsible for several forms of early-onset familial AD (FAD). *PSEN1* and *PSEN2* encode polytopic transmembrane proteins of 467 and 448 amino acids, respectively, which show about 65% sequence identity between the two proteins. While PS1 expression level is higher than that of PS2, both proteins are expressed ubiquitously in the brain and peripheral tissues of adult mammals (Lee *et al.* 1996). The model for PS with eight or nine transmembrane domains is generally accepted and PS has a hydrophilic loop domain between the putative 6th and 7th transmembrane domains facing the cytoplasm (Doan *et al.* 1996) and is cleaved by an unidentified protease within this loop resulting into two fragments, N- and C-terminal fragment (NTF and CTF), that remain associated as a heterodimer (Thinakaran *et al.* 1996). This proteolytic cleavage is thought to occur when nascent PS assembles with NCT, Aph-1, and Pen-2 as a γ-secretase complex and activates PS as the catalytic component of aspartyl protease (Iwatsubo 2004; Kopan and Ilagan 2004; Selkoe and Wolfe 2007).

The single-pass membrane protein NCT may recognize the substrate proteins of γ-secretase (Yu *et al.* 2000; Shah *et al.* 2005). The extracellular domain of NCT resembles an aminopeptidase, but lacks catalytic residues, and can interact with the N-terminal stubs of γ-

secretase substrates after ectodomain shedding (Shah *et al.* 2005). Thus, shedding of membrane proteins may be essential for the production of free N-termini of these proteins retained in the membrane, which can then be recognized by NCT. Aph-1 is thought to act as a scaffold during the process of γ-secretase complex assembly, and Pen-2 was suggested to act as a trigger for the proteolytic cleavage of PS to regulate PS activity (Kopan and Ilagan 2004; Selkoe and Wolfe 2007).

3.2 Processing mechanisms of several γ-secretase substrates, such as APP, are very similar to that of Notch

Precise steps of Notch processing are recently made clear (Fig.3). After translation, Notch is cleaved by furin-like covertase at the S1 site in the *trans*-Golgi network, and the two resulting fragments remain associated to form a functional heterodimer that is expressed on the cell surface (Logeat *et al.* 1998).



(A) In response to ligand binding, Notch undergoes shedding due to metalloprotease cleavage at the S2 site within the JM domain. After shedding the extracellular domain, the remaining Notch stub is further cleaved by γ-secretase at S3 and S4 sites within the TM domain. This sequential proteolysis produces NICD and Nβ fragment. (B) Cleavage of APP by α-secretase or β-secretase at the α-site or β-site, respectively, within the JM domain results in shedding of almost the entire extracellular domain and generates membrane-tethered α- or β-carboxy terminal fragments (CTFs). Several zinc metalloproteinases and BACE2 can cleave APP at the α-site, while BACE1 cleaves APP at the β-site. After shedding the extracellular domain, the remaining stub is further cleaved at least twice within the TM domain at γ- and ε-sites by γ-secretase, producing either p3 peptide (in combination with α-secretase) or Aβ (in combination with BACE1), respectively, and AICD. (C) Several stimuli, such as PKC activation and Ca^{2+} influx, trigger ectodomain cleavage of CD44 by a metalloprotease at the site within the JM domain, resulting in the secretion of soluble CD44 (sCD44). After shedding the extracellular domain, the remaining CD44 stub is further cleaved by γ-secretase at two sites within the TM domain. This sequential proteolysis produces the CD44 ICD and CD44β, an Aβ-like peptide.

Fig. 3. Similarities in the proteolytic processes among Notch, APP, and CD44.

As mentioned above, the sequential proteolytic cleavage called RIP mechanism is initiated by ligand binding and shedding at the S2 site by TACE or ADAM protease making the truncated Notch (Pan and Rubin 1997; Brou *et al.* 2000). Truncated Notch is further cleaved by γ-secretase in at least two sites within the TM domain, *i.e.*, at the S3 site to release NICD and at the S4 site to release the remaining small peptide (Nβ) (Kopan *et al.* 1996; Schroeter *et al.* 1998; Okochi *et al.* 2002), which resembles Aβ.

The proteolytic process of APP resembles that of Notch and also follows the RIP mechanism (Fig.3). Cleavage of APP by α-secretase (Esch *et al.* 1990) or β-secretase (Vassar *et al.* 1999) at the α-site or β-site, respectively, within the JM region results in shedding of almost the entire extracellular domain and generates membrane-tethered α- or β- CTFs. Several zinc metalloproteinases (Buxbaum *et al.* 1998; Lammich *et al.* 1999) and the aspartyl protease BACE2 can cleave APP at the α-site (Farzan *et al.* 2000), while BACE1 (β-site APP cleaving enzyme) cleaves APP at the β-site (Vassar *et al.* 1999). After shedding, the remaining stub is further cleaved at least twice by γ-secretase within the TM domain at γ- and ε-sites resulting in production of either non-amyloidogenic p3 peptide (in combination with α-secretase) or amyloidogenic Aβ (in combination with BACE1), respectively, and AICD (Kopan and Ilagan 2004; Selkoe and Wolfe 2007). In addition, AICD was shown to be a substrate of caspase and to be cleaved at the group III caspase consensus sequence 16 amino acids from the membrane border within the AICD.

It has been reported that several γ-secretase substrates also follow the RIP mechanism with release of their ICDs from the cell membrane. As shown in Fig. 3, the process of sequential proteolytic cleavage of CD44, which is important for immune system function, is very similar to those of Notch and APP and follows the RIP mechanism (Nagase *et al.* 2011). In addition, the ICD of this protein (CD44ICD) is also translocated to the nucleus, suggesting that CD44ICD may also mediate the gene expression.

3.3 Does γ-secretase mediate signaling events of type 1 transmembrane proteins?

γ-Secretase seems to cleave a diverse set of type1 transmembrane proteins, which have been shed their extracellular domains, in a sequence-independent manner (Struhl and Adachi 2000). As reflected by the flexible sequence specificity of γ-secretase activity, more than 50 type 1 transmembrane proteins have been reported as substrates of γ-secretase (McCarthy *et al.* 2009). As shown in Table 1, these substrates also have a wide range of functions, including roles in cell differentiation (Notch, Delta, and Jagged), cell adhesion (N-cadherin, E-cadherin, and CD44), synaptic adhesion (Nectin-1α), ion conductance regulation (voltage-gated sodium channel β2 subunit), axon guidance and tumor suppression (DCC), neurotrophin receptor (P75NTR), and its homolog (NRADD), lipoprotein receptor (ApoER2), and growth factor-dependent receptor tyrosine kinase (ERBB4).

As mentioned above, proteolytic cleavages of several γ-secretase substrates, such as APP and CD44, follow the RIP mechanism. The ICDs of these substrates are released from the cell membrane to cytoplasm by γ-secretase, and finally these ICDs translocate to the nucleus. These processes are very similar to those involved in Notch signaling. Thus, the observations that the common enzyme, γ-secretase, modulates proteolysis and the turnover of possible signaling molecules led to the signaling hypothesis suggesting that mechanisms similar to those

occurring in the Notch signaling pathway may contribute widely to γ-secretase-regulated signaling pathways (Koo and Kopan 2004; Nakayama *et al.* 2008a; Nakayama *et al.* 2011).

Indeed, as mentioned above, Dll1 is cleaved sequentially by proteases, probably including ADAM and γ-secretase, and Dll1IC is released from the cell membrane and undergoes translocation to the nucleus (Hiratochi *et al.* 2007). In the nucleus, Dll1IC enhances transcription of specific genes through binding to Smads. These observations suggest that Dll1 may also have a signaling mechanism similar to Notch signaling.

Substrate	**Function**	**PS or ICD function**
ApoER2	Lipoprotein receptor, neuronal migration	Activate nuclear reporter
APP	Precursor to Aβ, adhesion, trophic properties, axonal transport?	Aβ generation, release of ICD, complex with Fe65/Tip60, Cell death?
APLP1/2	Cell adhesion?	Form complex with Fe65 and Tip60
E-cadherin	Cell adhesion	Promote disassembly of adhesion complex
N-cadherin	Cell adhesion	Promote CBP degradation
β-catenin	Transduce Wnt signals stabilize adherens junctions	Facilitate phosphorylation
CD43	Signal transduction	Signaling molecule?
CD44	Cell adhesion	Activate TRE-mediated nuclear transcription
CSF1-R	Protein tyrosine kinase	Unknown
CXCL16 & CX3CL1	Membrane chemokine ligands	Unknown
DCC	Axon guidance, tumor suppressor	Activate nuclear reporter
Delta	Notch ligand	Transcription regulation
ERBB4	Receptor tyrosine kinase	Regulate heregulin-induced growth inhibition
HLA-A2	MHC class I molecule	Unknown
IGIF-R	Receptor tyrosine kinase	Unknown
IFN-αR2	Subunit of type I IFN-α receptor	Transcriptional regulation
IL-1RI	Cytokine receptor	Unknown
IL-1RII	Cytokine receptor	Unknown
Jagged	Notch ligand	Modulate AP-1 mediated transcription
LDLR	Lipoprotein receptor	Unkown
LRP	Scavenger and signaling receptor	Activate nuclear reporter
Na channel β-subunit	Cell adhesion, an auxiliary subunit of voltage-gated Na channel	Alter cell adhesion and migration
Nectin-1α	Adherens junction, synapse receptor	Remodeling of cell junctions?

Substrate	Function	PS or ICD function
Notch1-4	Signaling receptor	Transcription regulation
NRADD	Apoptosis in neuronal cells	Modulate glycosylation/matutaion of NRADD
P75NTR	Neurotrophin co-receptor, dependence receptor	Modulate p75-TrkA complex? Nuclear singaling?
γ-protocadherin	Cell adhesion, neuronal differentiation	Regulation of gene transcription?
Syndecan-3	Cell surface proteoglycan co-receptor	Regulation of membrane-targeting of CASK
Telencephalin	Cell adhesion	Turnover of telencephalin
Tyrosinase, Tyrosinase-related protein 1/2	Pigment synthesis	Intracellular transport of Post-Golgi Tyr-containing vesicles

PS, presenilin; ICD, intracellular domain; APLP, APP like protein; CBP, CREB (cAMP-responsive element binding protein)-binding protein; TRE, TPA (12-o-tetradecanoylphorbol 13-acetate)-responsive element; AP-1, activator protein-1; CASK, calmodulin-dependent serine kinase; Tyr, Tyrosinase.

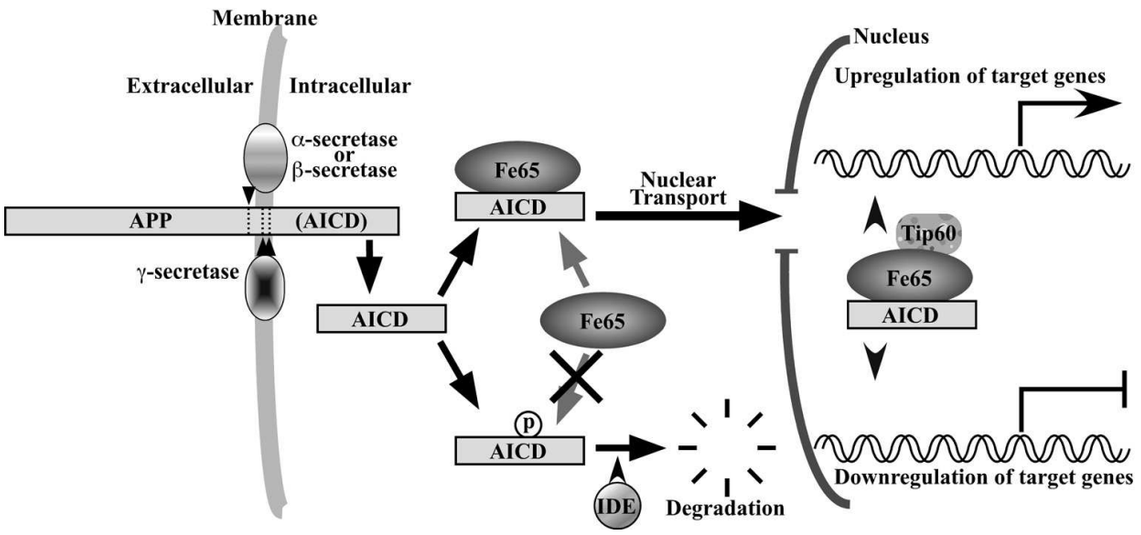
Table 1. Substrates for γ-secretase

3.4 Is γ-secretase a proteasome of the membrane?

As mentioned above, more than 50 type 1 membrane proteins have been reported as substrates of γ-secretase. This observation raises the simple question of why so many membrane proteins can transmit signals to the nucleus. In contrast to the signaling hypothesis, Kopan and Ilagan proposed another possibility that γ-secretase may act as a proteasome for membrane proteins (Kopan and Ilagan 2004). They pointed out that generally the ICDs of these substrates including AICD, which are released by γ-secretase, are rapidly degraded. Moreover, ectodomain shedding seems to be constitutive for some substrates, and ligand binding has been reported to enhance only intramembrane cleavage of Notch (Schroeter *et al.* 1998), Delta (Hiratochi *et al.* 2007), Syndecan-3 (Schulz *et al.* 2003), and ERBB4 (Ni *et al.* 2001). In addition, they also pointed out that the most evidence supporting the signaling hypothesis was obtained in overexpression experiments that differ somewhat from physiological conditions. Based on these observations, they proposed the proteasome hypothesis that the primary function of γ-secretase is to facilitate the selective disposal of type 1 membrane proteins (Kopan and Ilagan 2004).

While the proteasome hypothesis of γ-secretase is reasonable, there is no doubt that γ-secretase regulates signaling pathways of some substrates, such as Notch (Artavanis-Tsakonas *et al.* 1999; Selkoe and Kopan 2003; Koo and Kopan 2004). Although further studies are required to elucidate this issue, it is likely that γ-secretases are not uniform complexes but that different γ-secretase complexes may exist in different combinations with components such as Aph-1, Pen2, and/or PS isoforms, with different cellular functions, such as roles in signaling or degradation (Kopan and Ilagan 2004). Since γ-secretase substrates such as APP are generally more abundant than transcription factors, which are usually rare molecules, it is uncertain whether the majority of the ICDs of these substrates released by γ-secretase are required for the signaling mechanisms (Nakayama *et al.* 2008a; Nakayama *et al.*

2011). Although a large proportion of ICDs of these substrates are rapidly degraded, it is likely that a small amount of the remaining ICDs may be suitable for their functions with a small quantity of transcription factors. Thus, the greater part of ICDs of these substrates may be degraded and only a small proportion may play a role in signaling.



After cleavege of JM domain by α- or β-secretase, AICD is released from the membrane by γ-secretase. Non-phosphorylated AICD binds to the nuclear adaptor protein Fe65, which is thought to be essential for translocation of AICD to the nucleus, and forms complexes, alone or with the histone acetyltransferase Tip60. These complexes can immediately translocate to the nucleus, where they meidate up- and downregulation of certain target genes in association with Tip60. On the other hand, phospholylated AICD cannot translocate to the nucleus due to the inhibition of binding to Fe65, leading to rapid degradation by the proteasome and/or insulin-degrading enzyme (IDE).

Fig. 4. Putative APP signaling pathway.

In relation to this issue, an attractive model has been proposed (Fig.4) (Buoso *et al.* 2011). Binding to nuclear adaptor protein Fe65 is thought to be essential for translocation of AICD to the nucleus. In this model, since non-phosphorylated AICD binds to Fe65 and forms complexes, these complexes can immediately translocate to the nucleus, where they control the expression of certain genes in association with the histone acetyltransferase Tip60. On the other hand, as other stimuli induce phosphorylation of AICD, which strongly inhibits binding to Fe65, AICD without Fe65 cannot translocate to the nucleus. Phosphorylated AICD left in the cytosol is rapidly degraded, most likely by the proteasome and/or insulin-degrading enzyme (IDE) (Edbauer *et al.* 2002). Indeed, it has been reported that when phosphorylated at Thr^{668} in the APP-695 isoform, AICD cannot bind to Fe65 (Kimberly *et al.* 2005).

4. APP signaling?

4.1 Overview of APP

APP was first identified as a cDNA cloned using a partial amino acid sequence of Aβ fragment from the amyloid plaque of AD brains (Kang *et al.* 1987). APP is a type 1 membrane protein expressed in many tissues, especially concentrated in the synapses of

neurons. In humans, the APP gene contains at least 18 exons in a total length of 240 kb (Yoshikai *et al.* 1990), and several alternative splicing isoforms of APP have been observed, differing mainly in the absence (APP-695 which is predominately expressed in neurons) or presence (APP-751 and APP-770) of a Kunitz protease inhibitor (KPI) domain located toward the N-terminus of the protein (Sisodia *et al.* 1993). As mentioned above, APP undergoes sequential proteolytic cleavage reactions to yield the extracellular fragment, intracellular fragment (AICD), and Aβ fragment located in the membrane-spanning domain, which is thought to be the main cause of the onset of AD.

While APP has central roles in AD (Hardy 1997; Selkoe 2001), the physiological functions of this protein also remain to be clarified (Zheng and Koo 2006). It has been reported that APP acts as a cell adhesion molecule for cell-cell interaction (Soba *et al.* 2005), and as a neurotrophic and/or synaptogenic factor (Hung *et al.* 1992; Bibel *et al.* 2004; Leyssen *et al.* 2005). In addition, the possibility that APP is a cell-surface receptor is interesting from the signaling perspective. Several evidences support this idea; *e.g.*, Aβ can bind to APP and thus may be a candidate ligand for APP (Lorenzo *et al.* 2000). It has also been reported that F-spondin (Ho and Sudhof 2004) and Nogo-66 receptor (Park *et al.* 2006) could bind to the extracellular domain of APP and regulate Aβ production. Furthermore, the extracellular domain of APP may potentially interact in *trans* suggesting that APP molecules can bind to each other (Wang and Ha 2004).

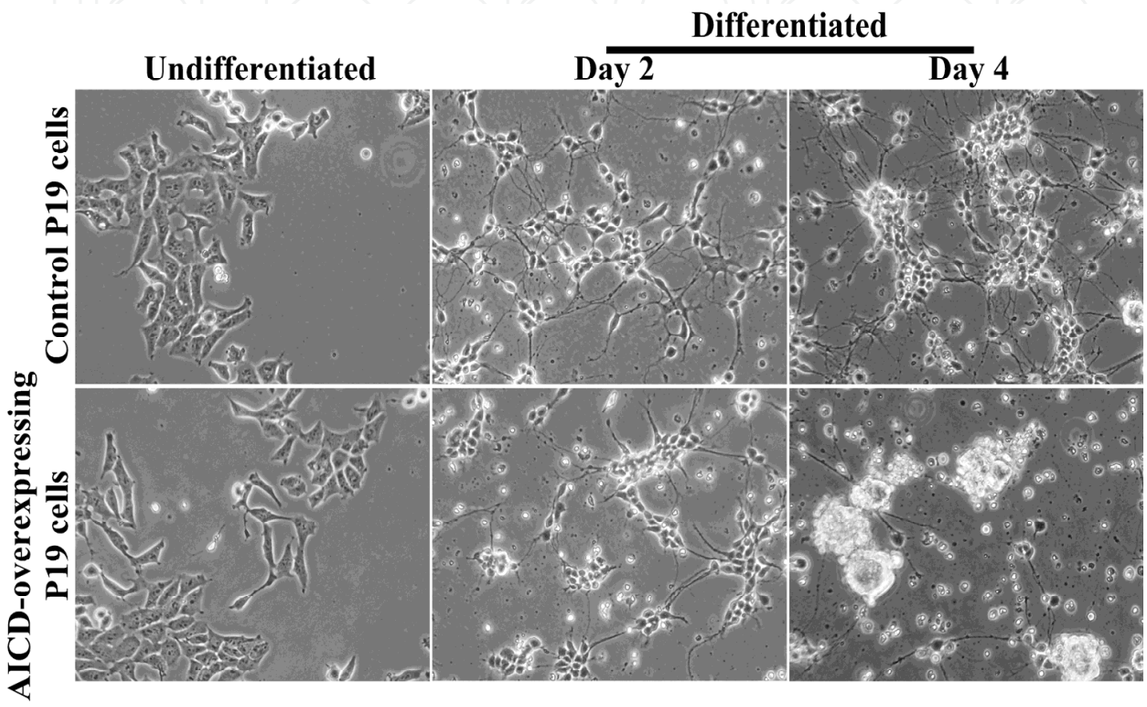
APP homologs show significant evolutionary sequence conservation in ICD (Nakayama *et al.* 2008b), which may reflect the functional importance of AICD. However, the Aβ region of this protein is not well conserved across species. As mentioned, AICD is thought to form complexes with Fe65 and these complexes translocate to the nucleus. In the nucleus, these complexes may associate with Tip60 and may bind to the *cis*-acting DNA sequence of the tetraspanin protein KAI1 gene to control transcriptional activity (Baek *et al.* 2002).

4.2 AICD induces neuron-specific apoptosis

There is accumulating evidence in support of the idea that APP signaling exists and contributes to the onset of AD. For example, transgenic mice overexpressing both AICD and Fe65 showed abnormal high activity of glycogen synthase kinase 3 beta (*Gsk3b* protein) (Ryan and Pimplikar 2005), leading to hyperphosphorylation and aggregation of TAU, resulting in microtubule destabilization, and reduction of nuclear β-catenin levels causing a loss of cell-cell contact mechanisms that may give rise to neurodegeneration in AD brain. In addition, it was also shown that c-Abl modulates AICD-dependent transcriptional induction, as well as apoptotic responses (Vazquez *et al.* 2009). Interestingly, elevated AICD levels have also been observed in AD brains (Ghosal *et al.* 2009). Therefore, it is highly possible that APP signaling changes expression patterns of certain genes and induces cell death, which may lead to AD pathology.

To explore APP signaling, we established several AICD-overexpressing embryonic carcinoma P19 cell lines (Nakayama *et al.* 2008b). Although neurons were differentiated from these cell lines after aggregation culture with RA treatment, AICD expression induced neuron-specific cell death. Indeed, while neurons from control cells which carried vector alone were healthy, almost all neurons from AICD-overexpressing P19 cells showed severe degeneration four days after induction of differentiation (Fig. 5). Moreover, DNA

fragmentation was detected, and all of terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP)-biotin nick end-labeling (TUNEL)-positive cells were also Tuj1-positive neurons. Based on these observations, we concluded that AICD can induce neuron-specific apoptosis (Nakayama *et al.* 2008b). The effects of AICD were restricted to neurons, with no effects observed on non-neural cells. Thus, although further studies are required, these results strongly suggest that AICD plays a role in APP signaling, which leads to the onset of AD.



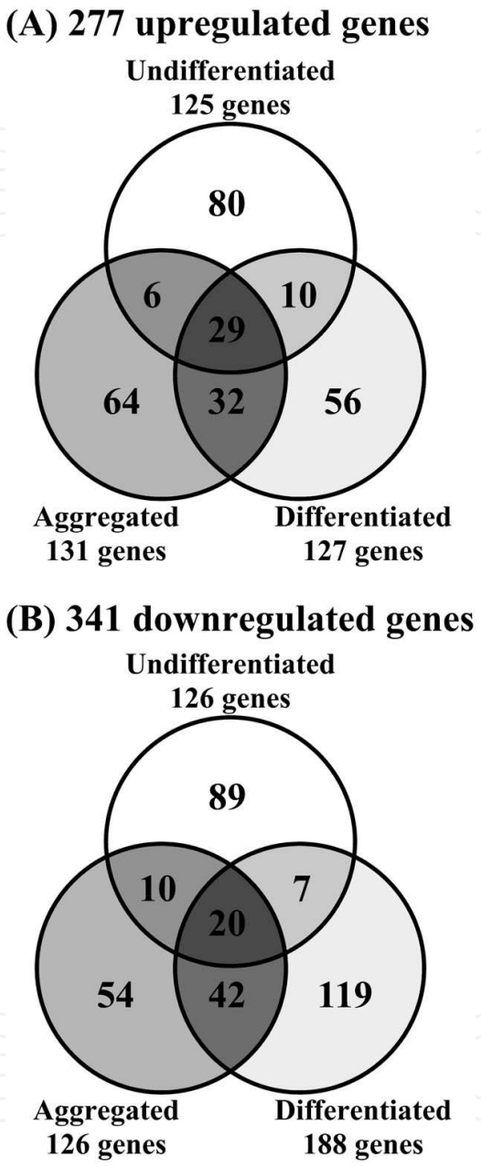
After aggregation culture with RA, AICD-overexpressing P19 and control P19 cells carrying vector alone were replated and cultured for the indicated periods on dishes and allowed to differentiate. Undifferentiated AICD-overexpressing P19 cells retained epithelial cell-like morphology similar to control cells, while the differentiated cells became round and showed a bipolar morphology with neurite extension. Two days after replating (Day 2), all cell lines grew well and neurons with long neurites appeared. Four days after replating (Day 4), control cells still grew well as clusters and many neurons had differentiated from these cells. However, many AICD-overexpressing P19 cells showed severe degeneration, becoming spherical with numerous vacuoles and detached from the culture dishes.

Fig. 5. Overexpression of AICD in P19 cells induces neuronal cell death.

4.3 AICD induces dynamic changes in the gene expression profile

If APP signaling exists, AICD should change expression of certain genes. To test this hypothesis and identify the genes involved in this process of neuron-specific apoptosis, we employed both AICD-overexpressing P19 cells and control P19 cells again and monitored AICD-induced changes in expressions of more than 20,000 independent genes by DNA microarray analysis at 3 time points during culture: the undifferentiated state, after 4 days of aggregation with RA (aggregated state), and 2 days after replating (differentiated state) (Ohkawara *et al.* 2011). Surprisingly, AICD can change expressions of a great many genes:

the expression levels of 277 genes were upregulated by more than 10-fold in the presence of AICD, while 341 genes showed downregulation of expression to less than 10% of the original level (Fig.6).



Venn diagrams showing the total numbers of genes upregulated by more than 10-fold in the presence of AICD (A) and genes downregulated to less than 10% of their original level (B) at three states of neural differentiation in P19 cells: undifferentiated, aggregated, and differentiated.

Fig. 6. Upregulated and downregulated genes by AICD.

AICD strongly induced expressions of several genes. For example, AICD-overexpressing P19 cells showed strong expression of protein tyrosine phosphatase receptor T (*Ptprt*) gene at all sampling points: 906-fold, 204-fold, and 116-fold upregulation, in undifferentiated, aggregated, and differentiated states, respectively, estimated from the intensity of

hybridization signals. In contrast to these upregulated genes, AICD also strongly inhibited the expression of several genes (Ohkawara *et al.* 2011). For example, *Hes5* was markedly increased through the process of neural differentiation: an increase of almost 300-fold in control P19 cells. However, this extreme induction in control P19 cells could not be detected in AICD-overexpressing P19 cells, indicating that AICD inhibits this induction. These results show that AICD induces both upregulation and downregulation of many genes, suggesting that AICD plays an important role in APP signaling.

We performed Gene Ontology (GO) analysis and classified these upregulated and downregulated genes according to GO terms (Ohkawara *et al.* 2011). While a few genes were classified into GO terms related to cell death, many genes were classified into GO terms unrelated to cell death. Furthermore, we evaluated AICD-induced changes in expression of genes thought to be involved in cell death in AD; however, we found no significant changes in expression of these genes. Therefore, it is likely that AICD does not directly induce the expression of genes involved in cell death, but the extreme dynamic changes in gene expression disrupt the homeostasis of certain neurons and thus give rise to neuron-specific cell death.

4.4 Amyloid hypothesis

Genetic studies indicate that both APP itself and its proteolytic processing are responsible for the onset of AD (Nakayama *et al.* 2011). The amyloid hypothesis is generally accepted as the mechanism of the onset of AD. The traditional amyloid hypothesis is that overproduced Aβ forms insoluble amyloid plaques, which are commonly observed in the AD brain and are believed to be the toxic form of APP responsible for neurodegeneration (Hardy and Selkoe 2002).

However, several issues have been raised regarding these hypotheses. One of the most significant arguments against the amyloid hypothesis is the presence of high levels of Aβ deposition in many non-demented elderly people (Terry RD 1999). This observation implies that Aβ amyloid plaques are not toxic. Based on these observations, the interesting possibility has been proposed that AD may be caused by an APP-derived protein, other than Aβ (Schnabel 2009). As both extracellular fragment and AICD are generated at the same time as Aβ, acceleration of proteolytic processing leads to overproduction of not only Aβ but also of both extracellular fragment and AICD. Therefore, it is likely that neuron-specific apoptosis induced by AICD may also be involved in the onset of AD.

5. Conclusion

Although γ-secretase plays central roles in AD, the physiological functions of this enzyme have yet to be fully elucidated. As reviewed here, Notch signaling is controlled by γ-secretase: intramembrane cleavage of Notch by γ-secretase serves to release ICD that has activity in the nucleus through binding to transcription factors. Recently, it was reported that many type 1 transmembrane proteins are substrates for γ-secretase, and ICDs of these substrates are released from the cell membrane by γ-secretase. These observations that the common enzyme, γ-secretase, modulates proteolysis and the turnover of possible signaling

molecules have led to the attractive hypothesis that mechanisms similar to the Notch signaling pathway may widely contribute to γ-secretase-regulated signaling pathways. Indeed, APP signaling induces dynamic changes in gene expression, which may be closely correlated with AICD-induced neuron-specific apoptosis and the onset of AD. Thus, it is likely that γ-secretase controls Notch signaling in NSCs and APP signaling in neurons that may lead to the onset of AD.

6. Abbreviations

AD	Alzheimer's disease
APP	amyloid precursor protein
AICD	the intracellular domain of APP
Aph-1	anterior pharynx defective-1
bHLH	basic helix-loop-helix
BMP	bone morphogenic protein
CTF	C-terminal fragment
Dll	Delta-like protein
Dll1IC	the intracellular domain of Dll1
EGF	epidermal growth factor
FAD	familial AD
GO	gene ontology
Hes	Hairy/Enhancer of split
ICD	intracellular domain
IDE	insulin-degrading enzyme
JM	juxtamembrane
Mib	Mind bomb
NCT	nicastrin
Ngn2	Neurogenin2
NICD	the intracellular domain of Notch
NSC	neural stem cell
NTF	N-terminal fragment
Pen-2	PS enhancer-2
PS	presenilin
RA	all-*trans*-retinoic acid
RIP	the regulated intramembrane proteolysis
TM	transmembrane

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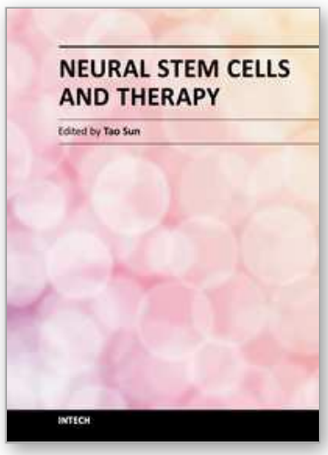

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This book is a collective work of international experts in the neural stem cell field. The book incorporates the characterization of embryonic and adult neural stem cells in both invertebrates and vertebrates. It highlights the history and the most advanced discoveries in neural stem cells, and summarizes the mechanisms of neural stem cell development. In particular, this book provides strategies and discusses the challenges of utilizing neural stem cells for therapy of neurological disorders and brain and spinal cord injuries. It is suitable for general readers, students, doctors and researchers who are interested in understanding the principles of and new discoveries in neural stem cells and therapy.

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